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**FIG. 1**

SEQ ID NO: 1:

GAGAAGGTTT GTTATGCCTC AGGGTTATCT GCAGTTCCC AATATTGACC CCGTATTGTT 60  
TTCGATCGGC CCTCTAGCGG TCGCCTGGTA TGGCTTGATG TATTGGTGG GTTCCCTTT 120  
TGCTATGTGG TTGGCCAATC GCCGAGCGGA TCGCGCGGC AGTGGTTGGA CGCGTGAGCA 180  
AGTCTCTGAC TTGTTATTG CCGGCTTTT AGGTGTAGTG ATCGGTGGCC GAGTTGGTTA 240  
TGTGATCTTC TACAATTTG ATCTGTTCCCT TGCTGACCCT CTTTATTTAT TCAAAGTGTG 300  
GACTGGCGGC ATGTCCTTCC AC GGCGGCTT ATTGGGTGTG ATCACCGCCA TGTTCTGGTA 360  
TGCGCGTAAA AACCAACGCA CCTTCTTG TGTTGCCGAT TTTGTTGCC CTTTAGTGCC 420  
ATTCGGTTTG GGGATGGGAC GTATCGGTAA CTTTATGAAT AGTGAACCTT GGGGACGAGT 480  
AACGGATGTG CCTTGGGCTT TTGTATTCCC TAATGGTGGC CCACTGCCGC GCCATCCTTC 540  
ACAGCTTAT GAATTCGCCT TAGAAGGCGT GGTTCTGTT CTTATTCTTA ATTGGTTTAT 600  
TGGTAAACCT CGTCCGCTAG GCAGCGTATC CGGACTGTTT TTAGCTGGAT ACGGTACATT 660  
CCGCTTCCTT GTGGAATACG TCCGTGAGCC AGATGCTCAG TTGGGTCTGT TTGGTGGCTT 720  
CATTCAATG GGGCAAATCC TCTCCTTACC TATGGTGATC ATCGGTATT TGATGATGGT 780  
TTGGTCTTAC AAGCGCGGTT TGTATCAAGA CCGTGTAGCA GCAAAATAGG GTAGTTAGGT 840  
GAAACAGTAT TTAGATCTT GTCAGCGCAT CGTCGATCAA GGTGTTGGG TTGAAAATGA 900  
ACGAACGGGC AAGCGTTGTT TGAATGTGAT TAATGCCGAT TTGACCTACG ATGTGGGCAA 960  
CAATCAGTTT CCTCTAGTGA CTACACGCAA GAGTTTTGG AAAGCTGCCG TAGCCGAGTT 1020  
GCTCGGCTAT ATTCTGGTT ACGATAATGC GGCGGATTTT CGCCAATTAG GTACCAAAAC 1080  
CTGGGATGCT AATGCCAATT TAAACCAAGC ATGGCTCAAC AATCCTTACC GTAAAGGTGA 1140  
GGATGACATG GGACCGCGTGT ATGGTGTCA GGGTAGAGCT TGGGCTAAGC CTGATGGTGG 1200  
TCATATTGAC CAGTTGAAAA AGATTGTTGA TGATTTGAGC CGTGGCGTTG ATGACCGAGG 1260  
TGAAATTCTT AACTTCTACA ATCCGGGTGA ATTCACATG GGGTGTGTC GCCCTTGCAT 1320  
GTACAGCCAT CATTTTCAT TGCTGGGGGA TACCTTGTAT CTCAACAGTA CTCAGCGTTC 1380  
ATGTGATGTG CCCTTGGGGT TGAATTCAA CATGGTGCAG GTTTATGTGT TCCTTGCCT 1440  
GATGGCACAG ATCACAGGGGA AAAAGCCGGG CTTGGCGTAT CACAAGATCG TCAATGCGCA 1500

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**FIG.1 (cont.)**

CATTTACCAA GATCAACTCG AATTGATGCG CGATGTGCAG CTAAAACGTG AGCCATTCCC 1560  
AGCGCCTCAG TTCCATATCA ATCCAAAGAT TAAAACACTG CAGGATTGG AAACCTGGGT 1620  
CACTTTGGAT GATTTGACG TCACCGGATA TCAGTTCCAC GATCCTATTG AATACCCGTT 1680  
TTCAGTCTAA TCCCGTATTG AGGCCGTATG CCTTGATGGG TTTTATATAA AAAAAGCTCC 1740  
CGAAGGTCGG GAGCTTTTT TATACAGATG ATGCTTAAC GCTTAAGCGG TTAGGGCAAG 1800  
AATGCTGCCG GGGATGACGA CAAACACACC CAATAAGTAA CTCACCACCA CCATTTGCT 1860  
CTTACAAGCC CAAGTTGAGA TGAGCTCAGC ACCTTTAATA GGCAAGTCGC GTAAGAAAGG 1920  
AATACCGTAA ATCAAGACCG TAGCCATCAA GTTAAAGCTT AAGTGCACCA GCGCAATTG 1980  
CAGAGCAAAC ACGGCAAAC CACCAAGAGAC AGCGGTTGCG GCGAGCAGAG CAGTAATACA 2040  
AGTGCCAATG TTCGCACCTA AGGTAAATGG GTAGATTCA CGCACTTCA GCACGCCAGA 2100  
GCCCACGAGA GGAACCATTG GGCTGGTTGT GGTCGATGAA GATTGAACCA ATACCGTAAC 2160  
CACTGTACCT GAAGCAATAC CGTGTAGTGG GCCTCGGCCA ATCGCATTGTT GTAGAATTTC 2220  
ACGTGCGCGG CCAACCATCA AACTCTCAT CAGTTGCCA ATCACCGTAA TGGCGACGAA 2280  
AATGGTCGCA ATACCCAATA CGATAAGTGC GACACCACCG AAAGTATTAC CCAATACCGA 2340  
AAGCTGGTT TCAAGCCCTG TGATGACAGG TTTGGTAATC GGTTTGATAA AATCAAAACC 2400  
TTTCATGCTC ATATGCCAG TCGCAAGCAG AGGCAGAACG AGCCAGTGTG AGACTTTCTC 2460  
TAAAATGCCA AACATCATTG CTAGAGGTAG GAAGATCAGC ACCCGAGAA GATTGAAAAA 2520  
ATCGTGGATG GTGGCACTGG CGAAAGCACG GCGAAACTCT TCTTACAGC GCATATGGCC 2580  
AAGGCTGACG AGAGTATTGG TCACAGTAGT ACCAATATTG GCACCCATCA CCATAGGAAT 2640  
CGCGGTTCA ACCGGTAACC CACCGAAC GAGACCAACA ATAATAGAAG TCACCGTGT 2700  
TGAGGATTGA ATCAGTGCAG TTGCCACTAA ACCAATCATC AATCCTGCAA TTGGGTGGGA 2760  
AGCAAATTCA AATAGAACTT TGGCTTGATC GCCGGTTGCC CATTAAAC CGCTGCCGAC 2820  
CATCGCACT GCAAGAAGTA GTAAATACAG CATGAAAGCC AAGTTGCCA AACGTAGGCC 2880  
TTTCGTGGTC AGCGAAATCG GCGCTGCAG 2909

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**FIG.2**

SEQ ID NO: 2:

GAGAAGGTTT GTTATGCCTC AGGGTTATCT GCAGTTCCC AATATTGACC CCGTATTGTT 60  
TTCGATCGGC CCTCTAGCGG TCGCGTGGTA TGGCTTGATG TATTTGGTGG GTTCCCTTT 120  
TGCTATGTGG TTGGCCAATC GCCGAGCGGA TCGCGCGGGC AGTGGTTGGA CGCGTGAGCA 180  
AGTCTCTGAC TTGTTATTG CCGGCTTTT AGGTGTAGTG ATCGGTGGCC GAGTTGGTTA 240  
TGTGATCTTC TACAATTTG ATCTGTCCT TGCTGACCCCT CTTTATTTAT TCAAAGTGTG 300  
GACTGGCGGC ATGTCCTTCC AC GGCGGCTT ATTGGGTGTG ATCACCGCCA TGTTCTGGTA 360  
TGC CGTAAA AACCAACGCA CCTTCTTGG TGTGGCCGAT TTTGTTGCC CTTTAGTGCC 420  
ATT CGGTTTG GGGATGGGAC GTATCGGTAA CTTTATGAAT AGTGAACCTT GGGGACGAGT 480  
AACGGATGTG CCTTGGGCTT TTGTATTCCC TAATGGTGGC CCACTGCCGC GCCATCCTTC 540  
ACAGCTTAT GAATT CGCCT TAGAAGGCGT GGTTCTGTT C TTTATTCTTA ATTGGTTAT 600  
TGGTAAACCT CGTCCGCTAG GCAGCGTATC CGGACTGTTT TTAGCTGGAT ACGGTACATT 660  
CCGCTTCCTT GTGGAATACG TCCGTGAGCC AGATGCTCAG TTGGGTCTGT TTGGTGGCTT 720  
CATTTCAATG GGGCAAATCC TCTCCTTACC TATGGTGATC ATCGGTATTT TGATGATGGT 780  
TTGGTCTTAC AAGCGCGGTT TGTATCAAGA CCGTGTAGCA GCAAAATAGG GTAGTTAG 838

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**FIG.3**

SEQ ID NO: 3:

TAATCCCGTA TTCAGGCGGT ATGGCTTGAT GGGTTTATA TAAAAAAAGC TCCCGAAGGT 60  
CGGGAGCTTT TTTTATACAG ATGATGCTTT AACGCTTAAG CGGTTAGGGC AAGAATGCTG 120  
CCGGGGATGA CGACAAACAC ACCCAATAAG TAACTCACCA CCACCATTTC GCTCTTACAA 180  
GCCCAAGTTG AGATGAGCTC AGCACCTTA ATAGGCAGTT CGCGTAAGAA AGGAATACCG 240  
TAAATCAAGA CCGTAGCCAT CAAGTTAAAG CTTAAGTGCA CCAGCGCAAT TTGCAGAGCA 300  
AACACGGCAA ACTCACCAGA GACAGCGGTT GCGGCGAGCA GAGCAGTAAT ACAAGTGCCA 360  
ATGTTCGCAC CTAAGGTAAA TGGGTAGATT TCACGCACCT TCAGCACGCC AGAGCCCACG 420  
AGAGGAACCA TTAGGCTGGT TGTGGTCGAT GAAGATTGAA CTAATACCGT AACCACTGTA 480  
CCTGAAGCAA TACCGTGTAG TGGGCCTCGG CCAATCGCAT TTTGTAGAAT TTCACCGTGC 540  
CGGCCAACCA TCAAACCTTT CATCAGTTG CCCATCACCG TAATGGCGAC GAAAATGGTC 600  
GCAATACCCA ATACGATAAG TGCGACACCA CCGAAAGTAT TACCCAATAC CGAAAGCTGG 660  
GTTTCAAGCC CTGTGATGAC AGGTTGGTA ATCGGTTGTA TAAAATCAAACCTTTCATG 720  
CTCATATCGC CAGTCGCAAG CAGAGGCAGA ACGAGCCAGT GTGAGACTTT CTCTAAAATG 780  
CCAAACATCA TTTCTAGAGG TAGGAAGATC AGCACCGCGA GAAGATTGAA AAAATCGTGG 840  
ATGGTGGCAC TGGCGAAAGC ACGGGCAAAC TCTTCTTAC AGCGCATATG GCCAAGGCTG 900  
ACGAGAGTAT TGGTCACAGT AGTACCAATA TTGGCACCCA TCACCATAGG AATCGCGGTT 960  
TCAACCGGTA ACCCACCGGC AACGAGACCA ACAATAATAG AAGTCACCGT GCTTGAGGAT 1020  
TGAATCAGTG CCGTTGCCAC TAAACCAATC ATCAATCCTG CAATTGGGTG GGAAGCAAAT 1080  
TCAAATAGAA CTTGGCTTG ATCGCCGGTT GCCCATTAA AACCGCTGCC GACCATCGCG 1140  
ACTGCAAGAA GTAGTAAATA CAGCATGAAA GCCAAGTTG CCCAACGTAG GCCTTCGTG 1200  
GTCAGCGAAA TCGGCGCTGC AG 1222

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**FIG.4**

SEQ ID NO: 4:

Val Lys Gln Tyr Leu Asp Leu Cys Gln Arg Ile Val Asp Gln Gly Val  
 1 5 10 15

Trp Val Glu Asn Glu Arg Thr Gly Lys Arg Cys Leu Thr Val Ile Asn  
 20 25 30

Ala Asp Leu Thr Tyr Asp Val Gly Asn Asn Gln Phe Pro Leu Val Thr  
 35 40 45

Thr Arg Lys Ser Phe Trp Lys Ala Ala Val Ala Glu Leu Leu Gly Tyr  
 50 55 60

Ile Arg Gly Tyr Asp Asn Ala Ala Asp Phe Arg Gln Leu Gly Thr Lys  
 65 70 75 80

Thr Trp Asp Ala Asn Ala Asn Leu Asn Gln Ala Trp Leu Asn Asn Pro  
 85 90 95

Tyr Arg Lys Gly Glu Asp Asp Met Gly Arg Val Tyr Gly Val Gln Gly  
 100 105 110

Arg Ala Trp Ala Lys Pro Asp Gly Gly His Ile Asp Gln Leu Lys Lys  
 115 120 125

Ile Val Asp Asp Leu Ser Arg Gly Val Asp Asp Arg Gly Glu Ile Leu  
 130 135 140

Asn Phe Tyr Asn Pro Gly Glu Phe His Met Gly Cys Leu Arg Pro Cys  
 145 150 155 160

Met Tyr Ser His His Phe Ser Leu Leu Gly Asp Thr Leu Tyr Leu Asn  
 165 170 175

Ser Thr Gln Arg Ser Cys Asp Val Pro Leu Gly Leu Asn Phe Asn Met  
 180 185 190

Val Gln Val Tyr Val Phe Leu Ala Leu Met Ala Gln Ile Thr Gly Lys  
 195 200 205

Lys Pro Gly Leu Ala Tyr His Lys Ile Val Asn Ala His Ile Tyr Gln  
 210 215 220

Asp Gln Leu Glu Leu Met Arg Asp Val Gln Leu Lys Arg Glu Pro Phe  
 225 230 235 240

Pro Ala Pro Gln Phe His Ile Asn Pro Lys Ile Lys Thr Leu Gln Asp  
 245 250 255

Leu Glu Thr Trp Val Thr Leu Asp Asp Phe Asp Val Thr Gly Tyr Gln  
 260 265 270

Phe His Asp Pro Ile Gln Tyr Pro Phe Ser Val  
 275 280

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**FIG. 5**

SEQ ID NO: 5:

Met Pro Gln Gly Tyr Leu Gln Phe Pro Asn Ile Asp Pro Val Leu Phe  
1 5 10 15

Ser Ile Gly Pro Leu Ala Val Arg Trp Tyr Gly Leu Met Tyr Leu Val  
20 25 30

Gly Phe Leu Phe Ala Met Trp Leu Ala Asn Arg Arg Ala Asp Arg Ala  
35 40 45

Gly Ser Gly Trp Thr Arg Glu Gln Val Ser Asp Leu Leu Phe Ala Gly  
50 55 60

Phe Leu Gly Val Val Ile Gly Gly Arg Val Gly Tyr Val Ile Phe Tyr  
65 70 75 80

Asn Phe Asp Leu Phe Leu Ala Asp Pro Leu Tyr Leu Phe Lys Val Trp  
85 90 95

Thr Gly Gly Met Ser Phe His Gly Gly Leu Leu Gly Val Ile Thr Ala  
100 105 110

Met Phe Trp Tyr Ala Arg Lys Asn Gln Arg Thr Phe Phe Gly Val Ala  
115 120 125

Asp Phe Val Ala Pro Leu Val Pro Phe Gly Leu Gly Met Gly Arg Ile  
130 135 140

Gly Asn Phe Met Asn Ser Glu Leu Trp Gly Arg Val Thr Asp Val Pro  
145 150 155 160

Trp Ala Phe Val Phe Pro Asn Gly Gly Pro Leu Pro Arg His Pro Ser  
165 170 175

Gln Leu Tyr Glu Phe Ala Leu Glu Gly Val Val Leu Phe Phe Ile Leu  
180 185 190

Asn Trp Phe Ile Gly Lys Pro Arg Pro Leu Gly Ser Val Ser Gly Leu  
195 200 205

Phe Leu Ala Gly Tyr Gly Thr Phe Arg Phe Leu Val Glu Tyr Val Arg  
210 215 220

Glu Pro Asp Ala Gln Leu Gly Leu Phe Gly Gly Phe Ile Ser Met Gly  
225 230 235 240

Gln Ile Leu Ser Leu Pro Met Val Ile Ile Gly Ile Leu Met Met Val  
245 250 255

Trp Ser Tyr Lys Arg Gly Leu Tyr Gln Asp Arg Val Ala Ala Lys  
260 265 270

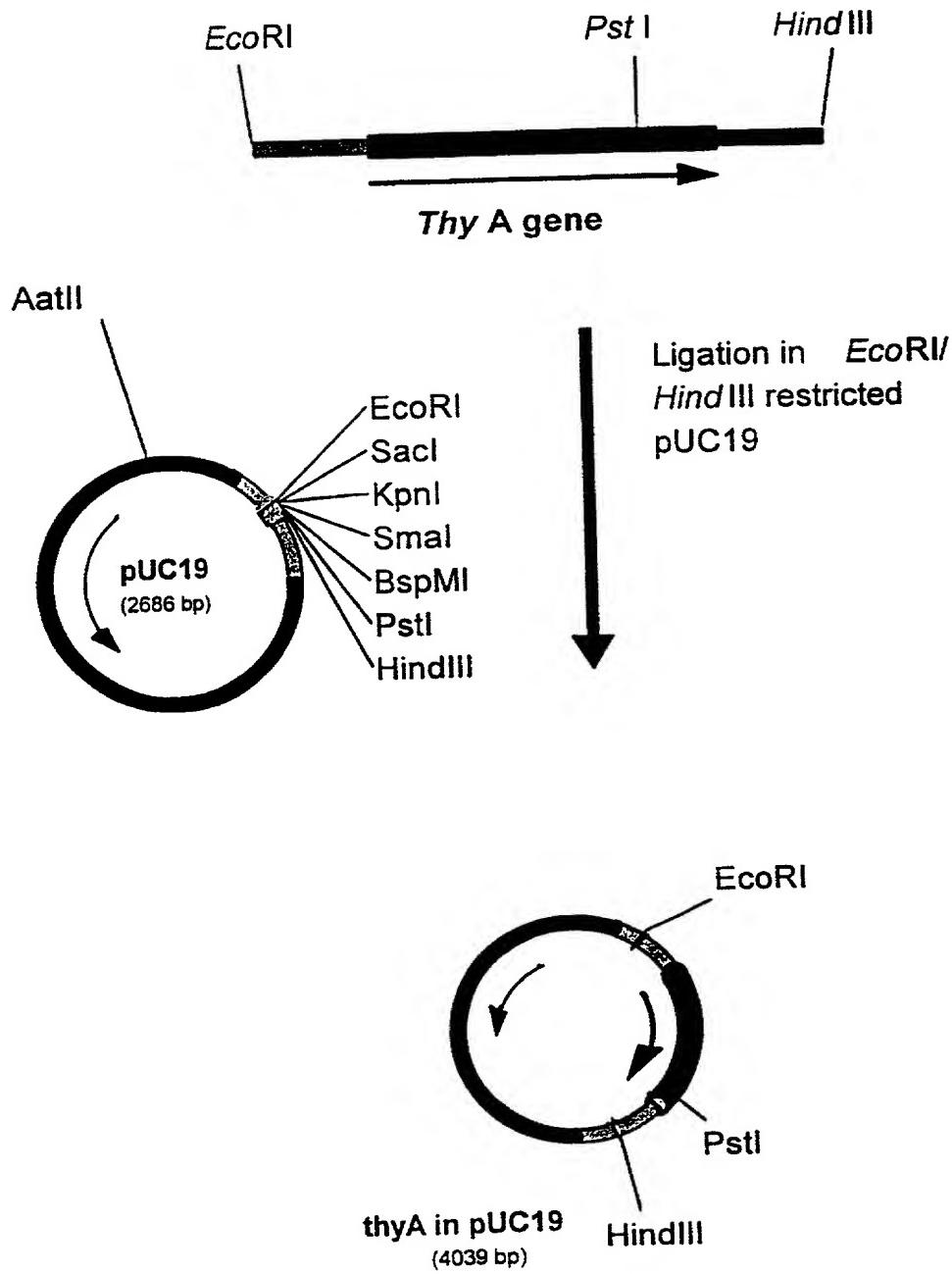
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FIG. 6



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## FIG. 7

E. coli : MKQYLELMQKVLDEGT-QKNDRRTGTTLSIFGHQMRFNL-QDGFPLVTTKRCHLRSIIHE  
V. cholerae : VKQYLDLCQRIVDQGVWVENERTGKRCLTVINADLYDVGNQFPLVTRKSFWKAIAE  
H. influenza: MKQYLELCRRIVSEGEWVANERTGKHCLTVINADLEYDVANNQFPLITTRKSYWKAIAE

E. coli : LLWFLQGDTNIAYHENNTIWD-----EWADE----NGDLGPVYGKOWRAWPTPDG  
V. cholerae : LLGYIRGYDNAADFRQLGKTWDANANLNQAWLNNSYRKGEDDMGRVYGVQGRAWKPDG  
H. influenza: FLGYIRGYDNAADFRALGKTWDANANENAALANPHRRGVDDMGRVYGVQGRAWRKPN

E. coli : RHIDQITTVLNQLKNDPDSRRIIVSAWNVGELDKMALAPCHAFFQFYVADGKLSCQLYQR  
V. cholerae : GHIDQLKKIVDDLSSRGVDDRGEILNPYNPGEFHMGCLRPMYSHHSLLGDTLYLNSTQR  
H. influenza: ETIDQLRKIVNNLTKGIDDRGEILTFNPGEFDLGCLRPMHTHTFSLVGDTLHLTSYQR

E. coli : SCDVFLGLPFNIAASYALLVHMMMAQQCDLEVGFVWTGGDTHLYSNHMD-QTHLQLSREPR  
V. cholerae : SCDVPLGLNFNMVQVYVFLALMAQITGKKPGLAYHKIVNAHIYQDQLELMRDVQLKREPF  
H. influenza: SCDVPLGLNFNQIQVFTFLALMAQITGKKAGKAYHKIVNAHIYEDQLELMRDVQLKREPF

E. coli : PLPKLIIKRKPESTFDY---RFEDFEIEGYDPHPGIKAPVAI  
V. cholerae : PAPQFHINPKIKTLQDLETWVTLDLDFVTGYQFHDPIQYPFSV  
H. influenza: PLPKELINPDIKTLLEDLETWVTMDDFKVVGYQSHEPIKYPFSV

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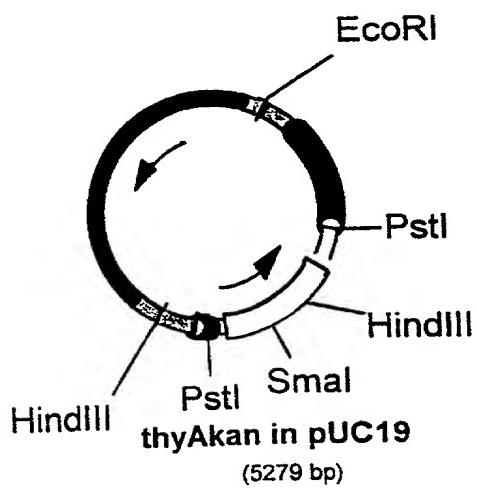
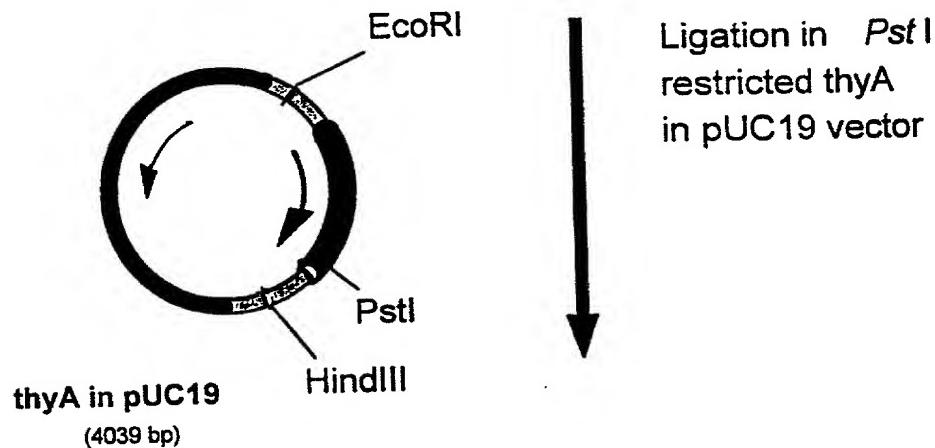
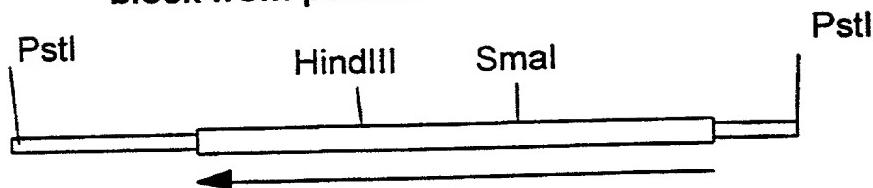
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FIG. 8

*PstI* restricted Kan gene  
block from pUC4K



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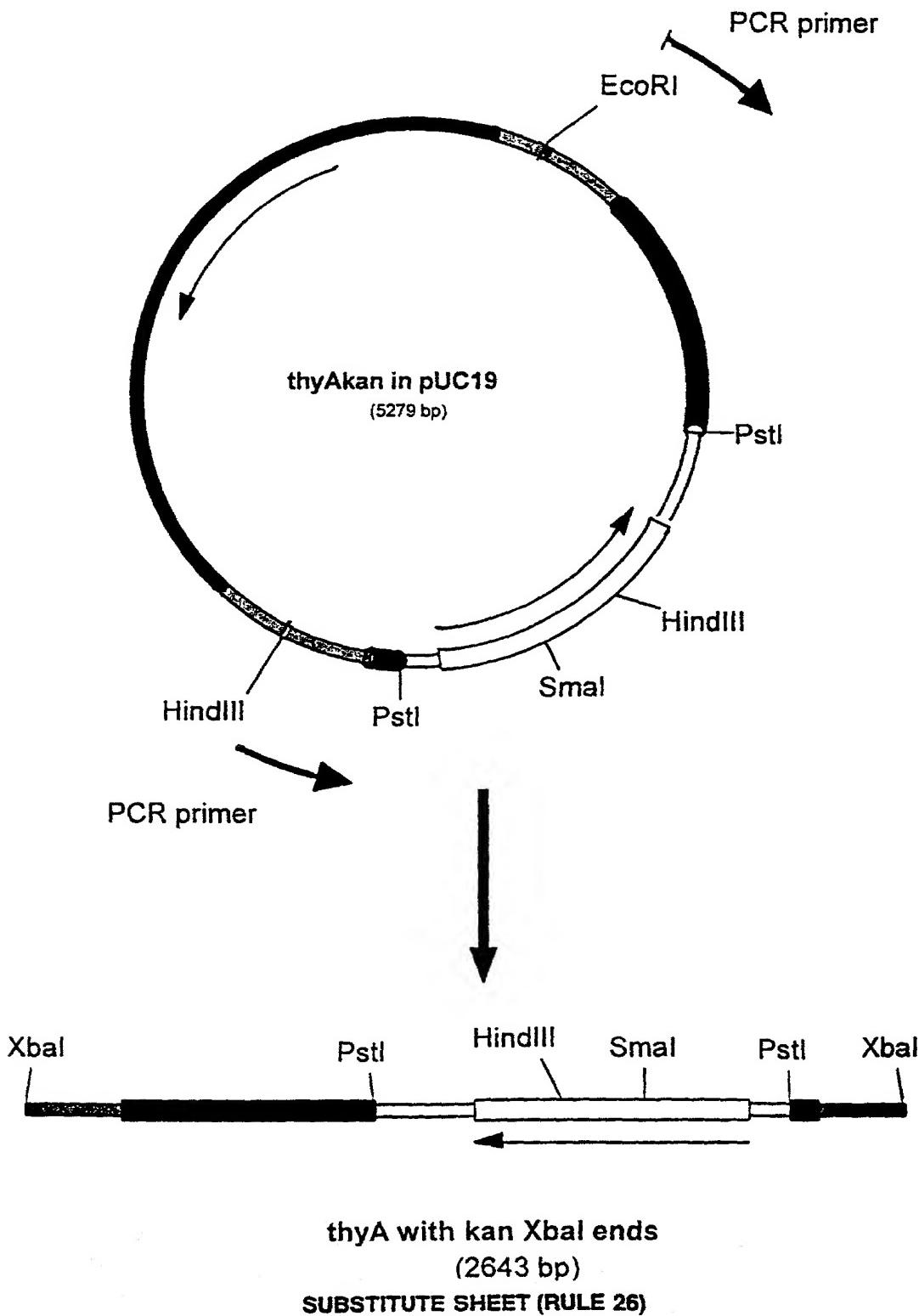
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FIG. 9

PCR to generate thyA-Kan-thyA fragment with *Xba*I ends.  
 Primers were chosen so that the *Eco*RI and *Hind*III sites  
 were eliminated



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FIG. 10

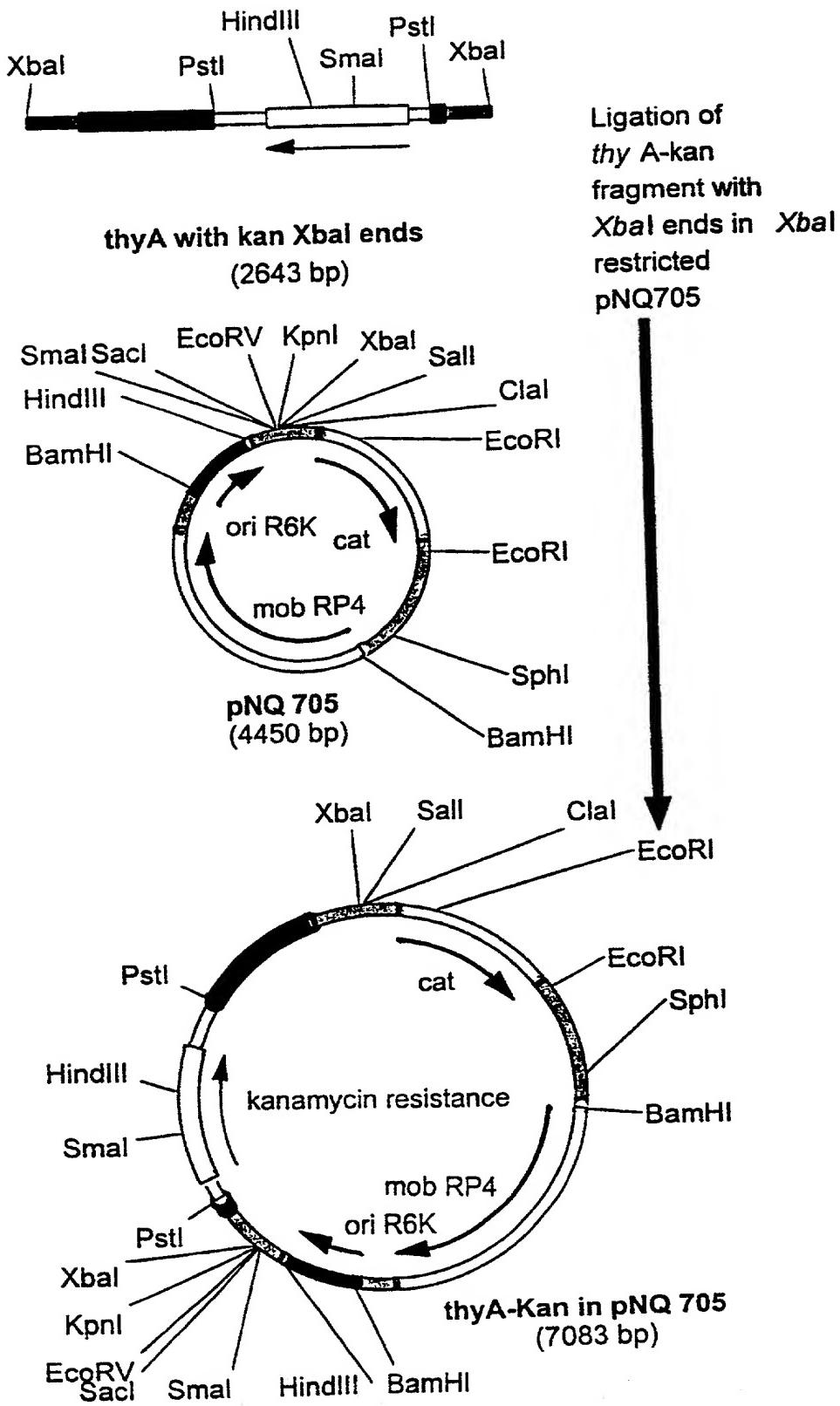
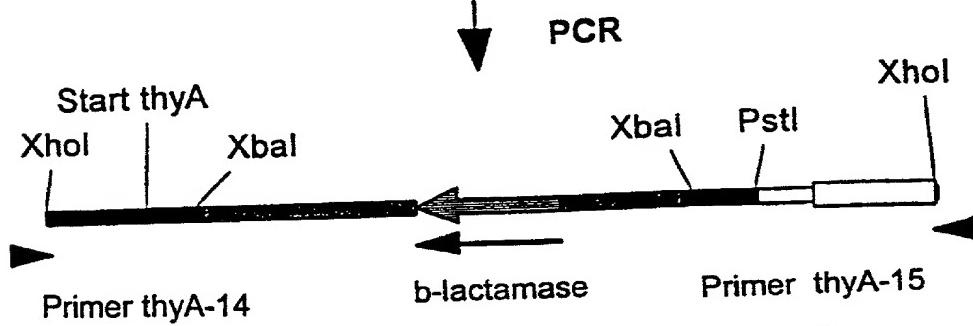
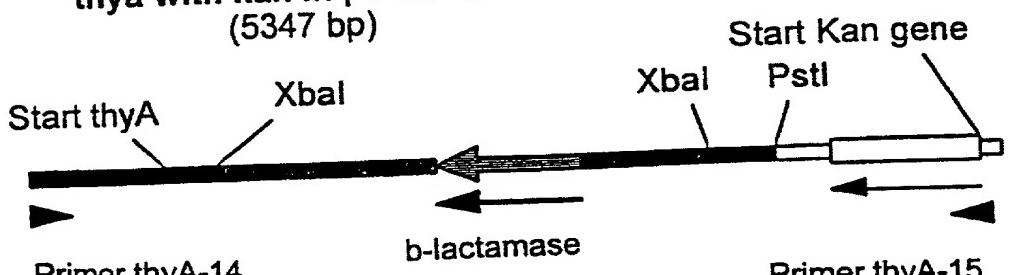
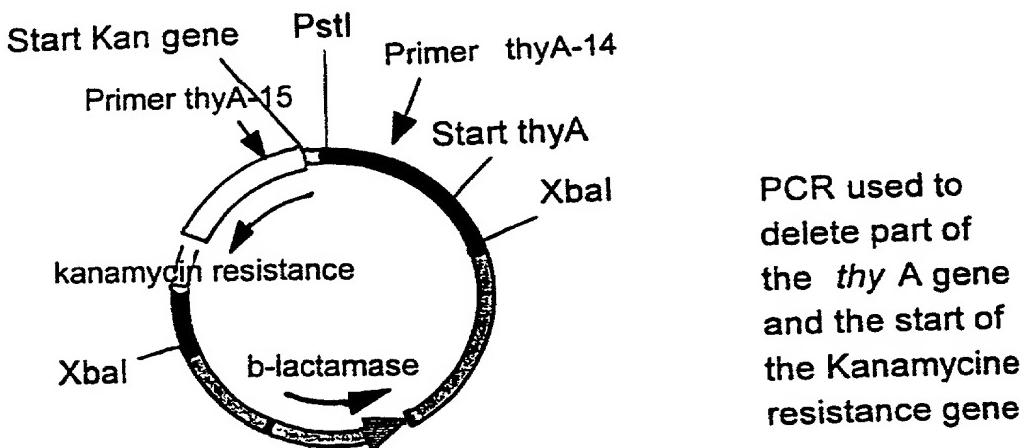
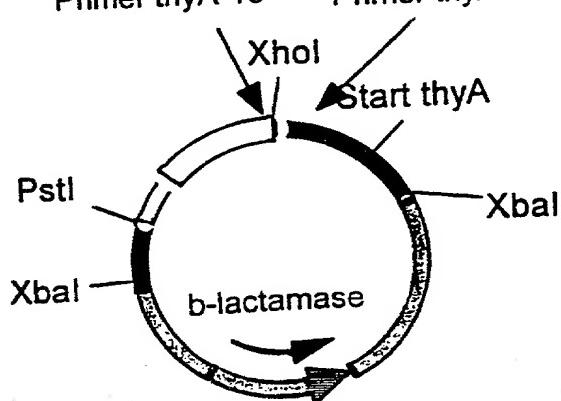


FIG. 11

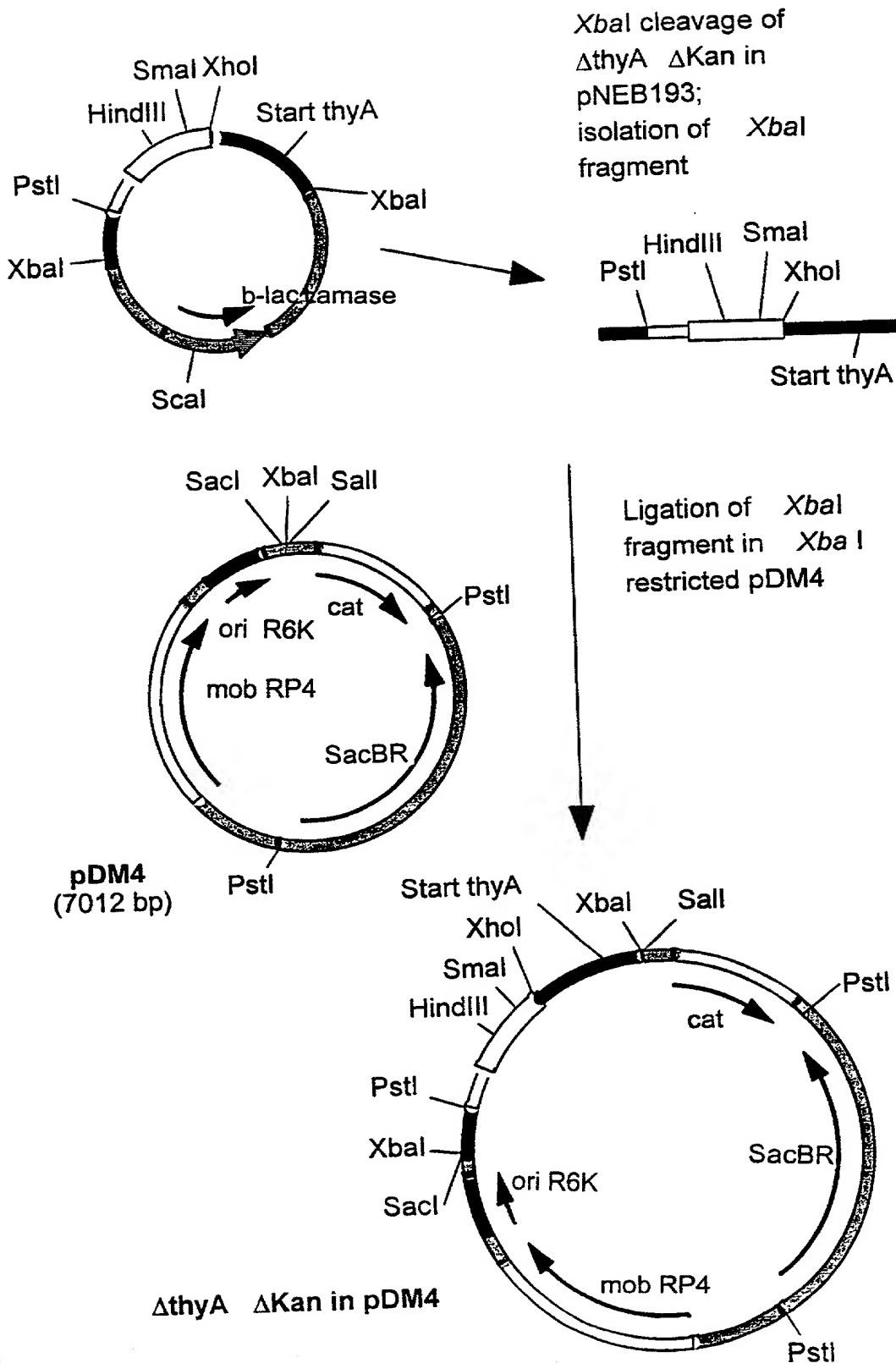


Cut with *Xhol* and self ligation

$\Delta$ thyA  $\Delta$ Kan in pNEB193 (4877 bp)



**FIG. 12**



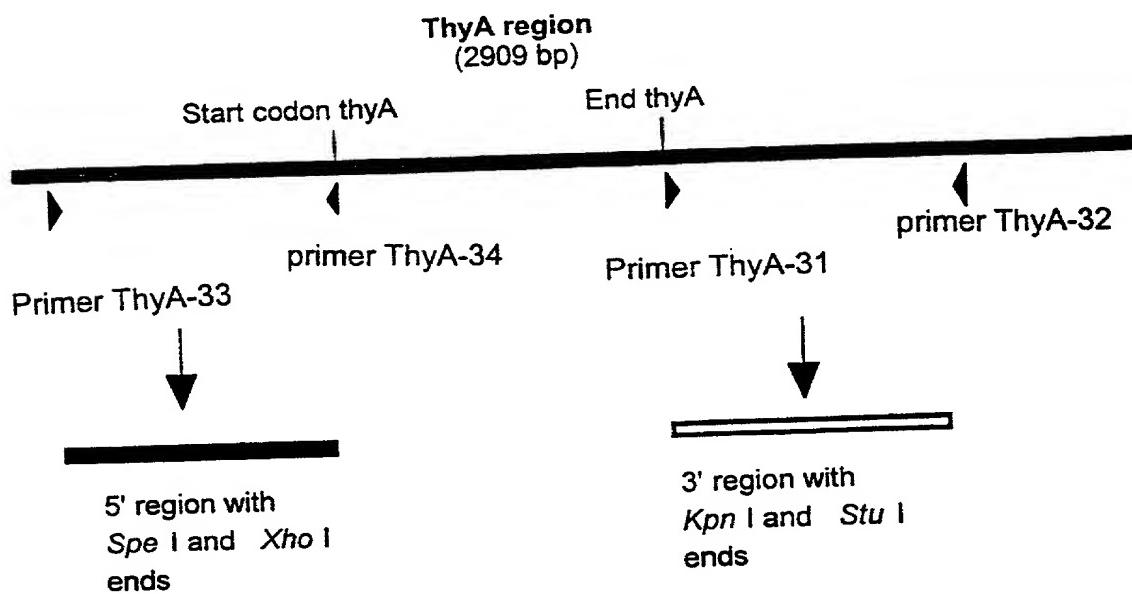
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FIG. 13



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FIG. 14

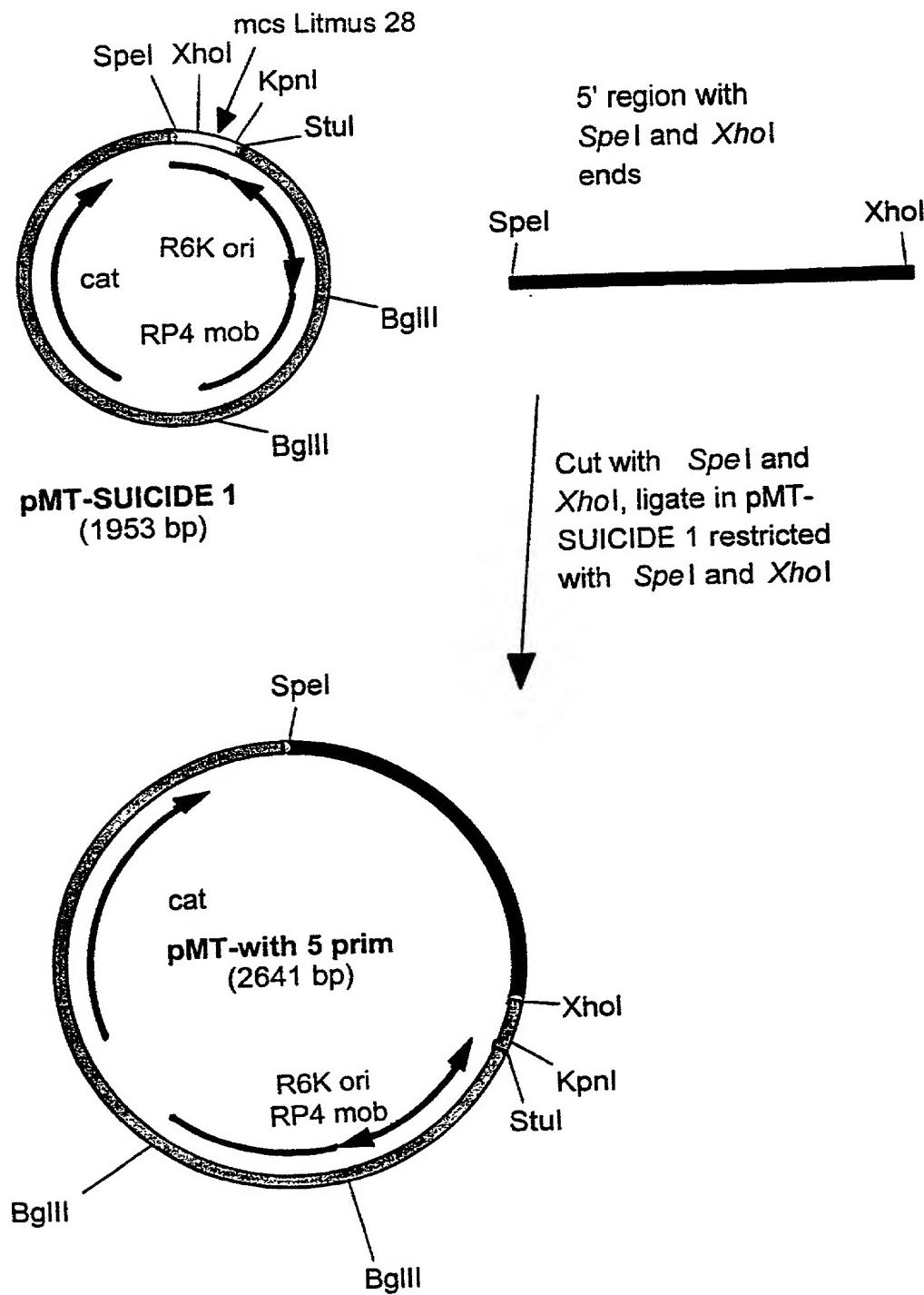
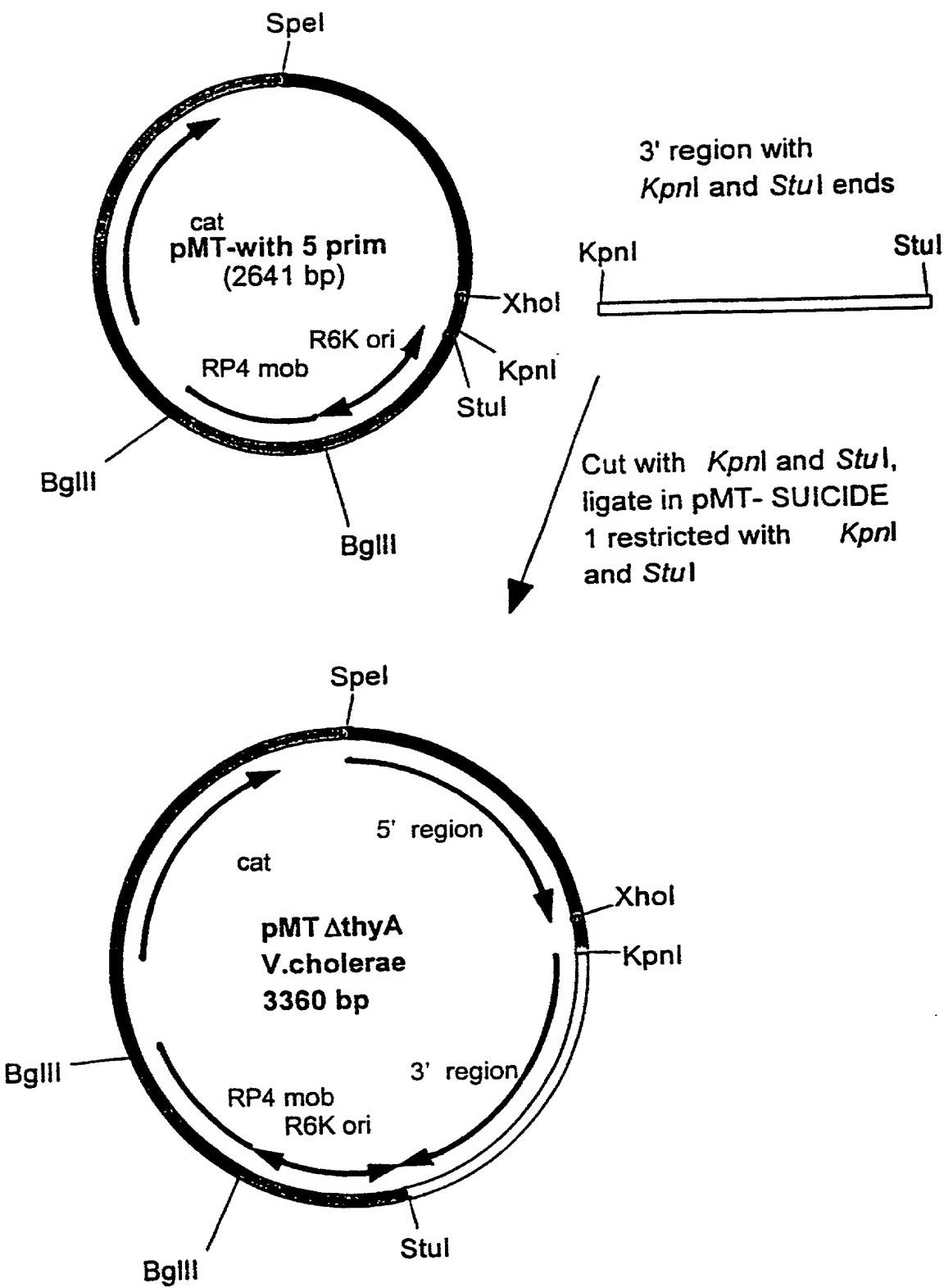


FIG. 15



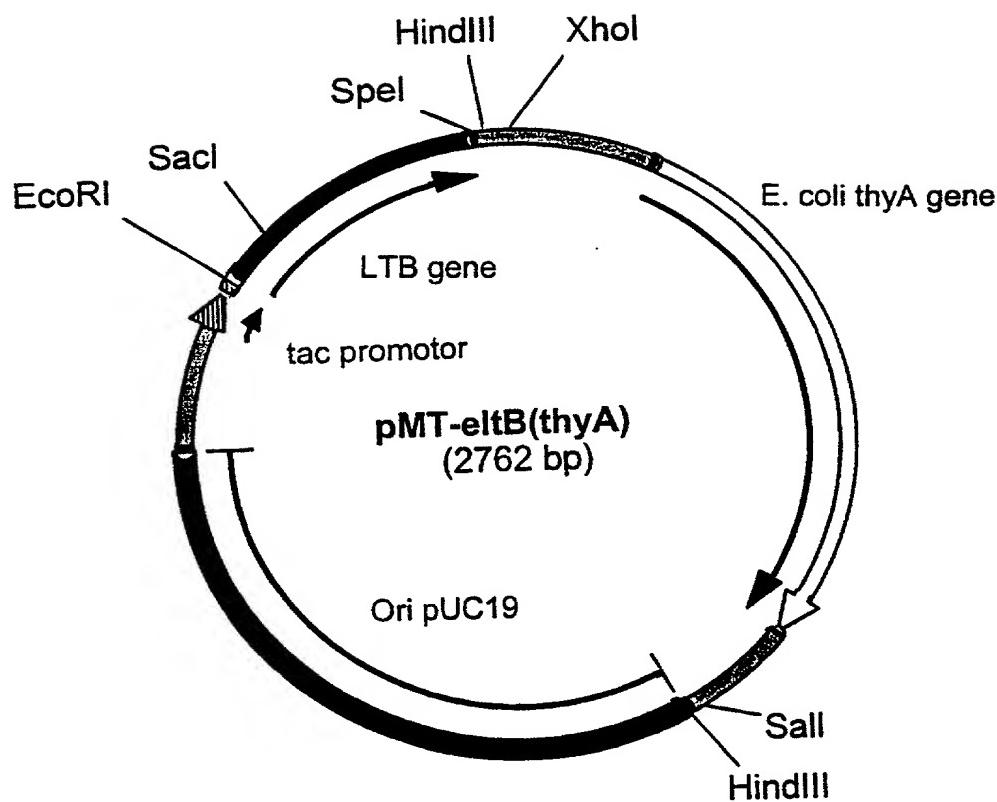
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**FIG. 16**



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FIG. 17

